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INTRODUCTION AND PURPOSE: Theoretically, decreasing hemoglobin oxygen affinity (HOA) of red blood cells (RBC) prior to transfusion should augment oxygen consumption during anemic hypoxia. Incubation of RBCs with phosphoenolpyruvate (PEP) increases 2,3-DPG and decreases HOA. This study evaluated the effect of transfusion with PEP treated RBCs on cardiovascular compensation and oxygen consumption in acutely anemic dogs.

MATERIALS AND METHODS: Anesthetized, splenectomized dogs were instrumented for continuous cardiovascular monitoring and underwent hemodilution to a hematocrit of 10%. After one hour, they were transfused to a hematocrit of 18% with either control or PEP treated stored blood. Vital signs and cardiac output (CO) were measured prior to hemodilution; vital signs, CO, and oxygen consumption were measured during severe anemia, and for one hour following transfusion.

RESULTS: Hemodilution to a HCT of 10% resulted in 100% increase of cardiac output. Transfusion with PEP treated blood resulted in increased oxygen consumption; transfusion with conventionally stored blood did not increase oxygen consumption, and cardiac output may have decreased more following PEP (79%) than control transfusion (98%).

CONCLUSION: Decreasing HOA results in improved oxygen consumption in the intact dog subjected to anemic hypoxia. Use of PEP treated blood has the potential to reduce transfusion requirements while ameliorating the cardiac compensation required by hemorrhage.

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INTRODUCTION

Since the relationship between 2,3-DPG and HOA was elucidated[1,2] the effects of altered HOA on oxygen availability and oxygen consumption have been studied. Sugarman et al originally described the negative effects of red cell 2,3-DPG depletion on oxygen consumption, and that depletion of high energy phosphorylated compounds has a negative effect on red cell survival and function.[3] Subsequently, various additives have been suggested to maintain both ATP and 2,3-DPG.[4] And while maintenance of ATP is possible, 2,3-DPG depletion is a consequence of all currently available liquid storage solutions [4,5].

Tomoda[6] and Hamasaki[7] showed that the phosphorylated glycolytic intermediate, phosphoenolpyruvate (PEP), can be transported across an intact red cell membrane, and accumulates intracellularly under specific conditions. In 1982, Sohmer and Scott [8] demonstrated that incubating stored human red blood cells (RBC) with PEP resulted in regeneration of 2,3-DPG up to two times the prestorage levels, with concomitant elevation of P_{50}.* They also found that PEP "amplified" RBCs increased oxygen consumption in an isolated perfused rat liver model of anemic hypoxia when compared to control red cells.[9] Since then, PEP has been suggested as an adjunct to liquid storage[10], and cells treated with PEP have been transfused into patients during coronary artery bypass surgery.[11] Although there was no observed toxicity, neither was there a difference in oxygen consumption between patients receiving PEP treated cells and those receiving conventionally stored blood. The hematocrit of

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* The partial pressure of oxygen (mmHg) at which hemoglobin is 50% saturated.

both groups was maintained above 30% however, and oxygen consumption was not limited by oxygen delivery at this relatively high hematocrit. The authors concluded that oxygen consumption had the potential to be improved using PEP treated cells. The present study was designed to evaluate the effect of transfusion with PEP treated RBC on oxygen consumption in the intact, but severely anemic dog.

MATERIALS AND METHODS

Mixed sex, adult dogs were used in this experiment (N=4 control; N=3 PEP). To prevent intraoperative "auto-transfusion" from a contractile spleen, they were splenectomized at least three weeks prior to the study. Following an overnight fast, the animals were anesthetized and intubated, anesthesia was maintained using 2-3% isoflurane throughout. Respiration was controlled at a minute volume of 100cc/kg and respiratory rate was adjusted between 6-8 breathes/min to maintain an arterial pCO_2 at 30-40 mm Hg. The dogs were instrumented for continuous monitoring of BP, HR, and rectal temp. A pulmonary artery catheter was placed via the external jugular vein for measurement of cardiac output, and mixed venous blood sampling. Oxygen consumption was determined using the Fick equation; arterial and mixed venous blood gas samples were analyzed with a Instrumentation Laboratory Blood gas analyzer.

Experimental Design: Following instrumentation and heparinization (200 units/kg), cardiac output at normal hematocrit was measured. Hemodilution was then performed, removing 2.5 cc/kg blood every 2 minutes with simultaneous replacement of volume using warmed 3.5% albumin in Lactated Ringers. Hemodilution to a hematocrit of 10% required 90-120 minutes, depending on the initial hematocrit (range 30-42%). Vital signs, cardiac output, and oxygen

consumption were measured at 0, 30, and 60 minutes of severe anemia. After this period, the dogs were transfused over 30-60 minutes to a hematocrit of 18 (range 16-20) with either control or PEP treated RBCs. Vital signs, cardiac output, and oxygen consumption and were again measured over one hour, and the animals were euthanized at the termination of the second experimental period.

Preparation of Blood: Canine blood collected sterilely in commercially available CPD solution (Citrate, Phosphate, Dextrose Preservation - Baxter Laboratories) and stored at 4 degrees from 5-21 days, was used throughout this experiment. Prior to transfusion, blood for the control group was centrifuged, washed twice with two volumes of iced, heparinized saline containing gentamicin at 20 mg/liter; the final hematocrit was adjusted to 55-65% with saline. Phosphoenolpyruvate treatment has been described previously.[8] Briefly, stored centrifuged red cell concentrate was mixed 1:1 (v:v) with a sterile 52 mM solution of PEP in CPD and incubated for 4 hours in a 37 degree shaking water bath. After completion of the 4 hour incubation, the cells were washed with two volumes of iced saline as above, and the final hematocrit adjusted. Blood for both groups was filtered using standard blood filters prior to transfusion. The P_{50} was derived from the hemoglobin oxygen dissociation relationship, which was determined using a Hemox Analyzer (TCS Medical Products).

RESULTS

Vital signs (BP, HR, Temp.) and the hematocrit of the animals throughout the experiment are presented in Table 1. Cardiac output of the dogs during the initial control period was found to be .137 + .021 liter/kg/min, well within the range reported for anesthetized dogs.[13,14] The average change in cardiac output caused by hemodilution and

transfusion is shown in Table 2. Both groups showed a marked increase after hemodilution, with a subsequent non-significant decrease following transfusion. Mixed venous oxygen content and change in percent saturation between arterial and venous blood is shown in Table 3. Oxygen consumption during anemia and following transfusion in both groups is shown in Table 4. Representative hemoglobin oxygen affinity dissociation curves of control and PEP treatment animals, both pre and post transfusion, are presented in Figures 1 and 2, respectively. Overlying the curves are the mean changes in percent saturation for both treatments, and the P_{50} is shown on each curve.

DISCUSSION

Hemodilution to a hematocrit of 10% resulted in doubling of cardiac output in the dog, similar to the results obtained by other groups using dogs,[12,13] As shown in Tables 2 and 4, transfusion with conventionally stored blood did not increase oxygen consumption or decrease cardiac output, despite increasing the hematocrit more than 80%. Similar results were obtained by Shah et al[14] and Fortune et al[15] in humans, who found that transfusion with stored blood in patients did not necessarily improve oxygen transport; they attributed this effect, at least partially, to the reduced P_{50} of the stored blood. With other factors (hematocrit, duration of anesthesia, pH) controlled in the present study, it seems likely that the increased oxygen affinity of the stored blood was responsible for preventing oxygen "off-loading" from the hemoglobin supplied by the control transfusions as shown by the marked reduction in the saturation difference (to 17%) between arterial and venous blood in the control post transfusion group. Although transfused blood eventually regenerates normal levels of 2,3-DPG, and has normal P_{50} by 24-36 hours post transfusion,[17] the requirement to increase oxygen consumption, or to maintain oxygen

consumption without increasing cardiac output or lowering mixed venous oxygen tension is usually more acute than 24 hours.

Transfusion with PEP treated (high P_{50}) blood yielded significantly greater oxygen consumption. There was no difference in any other factor determining oxygen consumption (pH, cardiac output, arterial or mixed venous oxygen tension, hematocrit) between the two treatments except HOA. The hemoglobin oxygen dissociation curves were, however, quite different, as shown in Figures 1 and 2. Earlier work in our lab showed that 1/3 exchange transfusion with PEP treated RBCs resulted in a change in the P_{50} of the intact animal, and that this effect was maintained for at least 6 hours.[8] Yonenaga et al later showed that the elevated P_{50} induced by transfusion with PEP treated blood lasted 48 hours post transfusion in dogs, and that PEP treated RBCs had normal in vivo survival.[16] The present experiment confirmed the ability of transfusion with PEP or conventionally stored RBCs to change the P_{50} of the blood of the whole animal following transfusion. The difference in oxygen consumption following transfusion with PEP treated cells in this study appears entirely due to alterations in the hemoglobin oxygen affinity; hematocrit was not different between the two groups, and cardiac output was clearly not greater in the PEP treated group, as shown in Table 2.

Increased red cell 2,3-DPG, with resultant increased P_{50} to enhance the availability of oxygen to the organism is a well documented physiologic response to the hypoxia of chronic anemia and high altitude.[12] Efforts to translate this finding to clinical medicine have been hampered by safe and efficacious methods to rapidly raise P_{50} either in vivo, or in vitro prior to transfusion. Phosphoenolpyruvate is a normal metabolite in mammalian cells and believed to be safe, but a prior study using PEP as a means to increase P_{50} did not show a beneficial effect on

oxygen consumption in patients receiving PEP treated blood. Oxygen consumption was demand, not delivery, limited in these patients however. These results of the present study of severe anemic hypoxia do support the concept of amplifying RBCs prior to transfusion as a means to immediately augment oxygen consumption following transfusion, with a possible lower total transfusion requirement.

REFERENCES

1. Benesch R, Benesch RE: The effect of organic phosphates from the human erythrocyte on the allosteric properties of hemoglobin. Biochem Biophys Res Comm 26:162, 1967.

2. Chanutin A, Curnish RR: Effect of organic and inorganic phosphates on oxygen equilibrium of human erythrocytes. Arch Biochem 96:121, 1967.

3. Sugarman HJ, Davidson AB, Sant Vibul, et al: The basis of defective oxygen delivery from stored blood. Surg Gynecol Obstet 131:733, 1970.

4. Vora S, West C, Beutler E: The effect of additives on red cell 2,3 diphosphoglycerate levels in CPDA preservatives. Trans 29(3):226-229, 1989.

5. Weed R, LaCelle P: ATP dependence of erythrocyte membrane deformability. In Greenwalt, TJ (ed.): Red Cell Membrane Structure and Function. Philadelphia, Lippincott, 1969.

6. Tomoda A, Hamasaki N, Minakami S: Transport of phosphoenolpyruvate through red cell membrane in acidified isotonic sucrose medium. Biochem Biophys Res Comm 66:1127, 1975.

7. Hamasaki N, Tomoda A, Harasaki H, Minakami S: Accumulation of phosphoenolpyruvate in red cells incubated with the phosphate ester in acidified isotonic sucrose medium. J Biochem 81:1505, 1977.

8. Sohmer P, Scott R: Phosphoenolpyruvate (PEP) effects on fresh and stored red blood cells. Proc Soc Exper Biol and Med 171:24-33, 1982.

9. Scott R, Sohmer P: Raising red cell P50 improves oxygen extraction by the isolated perfused liver. Shock 23:171, 1982.

10. Scott R, Sohmer P: Comparative effects of phosphoenolpyruvate on selected mammalian erythrocytes. Comp Biochem Biophys Acta 72A(4) 737-740, 1982.

11. Matsuzaki K, Takano H, Tokunaga K, Inaba S, Hamasaki N: Clinical application of phosphoenolpyruvate (PEP) to autologous transfusion of patients with open heart surgery. Fukuoka Acta Medica 84 (1):7-14, 1993.

12. Cain S: Oxygen delivery and uptake in dogs during anemic and hypoxic hypoxia. J Appl Physio 42(2):228-234, 1977.

13. Fan F, Chen R, Schuessler G, Chien S: Effects of hematocrit variations on regional hemodynamics and oxygen transport in the dog. Am Journ Physiol 238:H545-552, 1980.

14. Shah D, Gottlieb M, Rahm R, et al: Failure of red blood cell transfusion to increase oxygen transport or mixed venous PO2 in injured patients. J Trauma 22(9):741-746, 1982.

15. Fortune J, Feustel P, Saifi J, et al: Influence of hematocrit on cardiopulmonary function after acute hemorrhage. J Trauma, 27:243, 198717.

16. Yonenaga K, Todoroki H, Tokunaga K, Hamasaki N: Changes in adenosine triphosphate, 2,3-diphosphoglycerate and P_{50} of dog blood following transfusion of autologous red cells pretreated with phosphoenolpyruvate in vitro. Transfusion 26:194, 1986.

17. Lenfant C, Torrance JD, Reynafarje C: Shift of the oxygen-hemoglobin dissociation curve at altitude: mechanism and effect. J Applied Physiol 30:635-631, 1971.

TABLE 1

VITAL SIGNS AND HEMATOCRIT AT EACH PERIOD

	Pre-Bleed		Anemia		Post-Transfusion	
	Control	PEP	Control	PEP	Control	PEP
BP (mmHg)★	118/78	138/76	125/51	108/49	141/60	132/69
Heart Rate $\bar{X} \pm$ SEM	118 ± 10	115 ± 6	148 ± 10	148 ± 5	139 ± 12	132 ±69
Temp (C°) $\bar{X} \pm$ SEM	37.0 ± 3	37.7 ± .2	37.4 ± .3	37.6 ± .2	37.5 ± .3	38.0 ± 0.4
Hematocrit	38.4 ± 1.5	35.0 ± 3.0	10.0 ± 0.7	10.5 ± 0.6	17.4 ± 0.9	16.7 ± 0.6

★ mean systolic/mean diastolic

TABLE 2

EFFECT OF HEMORRHAGE AND TRANSFUSION ON CARDIAC OUTPUT

	Pre-Bleed liters/kg/min	Anemic Period liters/kg/min	Post Transfusion liters/ /kg/min
Control	.147 ± .018	.284 ± .033	.279 ± .030
PEP	.132 ± .034	.274 ± .009	.217 ± .027

TABLE 3

EFFECT OF PEP TRANSFUSION ON OXYGENATION

	Mixed Venous O_2		% SAT (A-V)	
	Pre (mmHg)	Post (mmHg)	Pre	Post ($Sa0_2$ - $Sv0_2$)
Control	38 ± 5	46 ± 4	32 ± 4	17 ± 2
PEP	37 ± 8	43 ± 11	37 ± 8	★47 ± 11

★ $P \leq .05$, Control vs. PEP, post transfusion

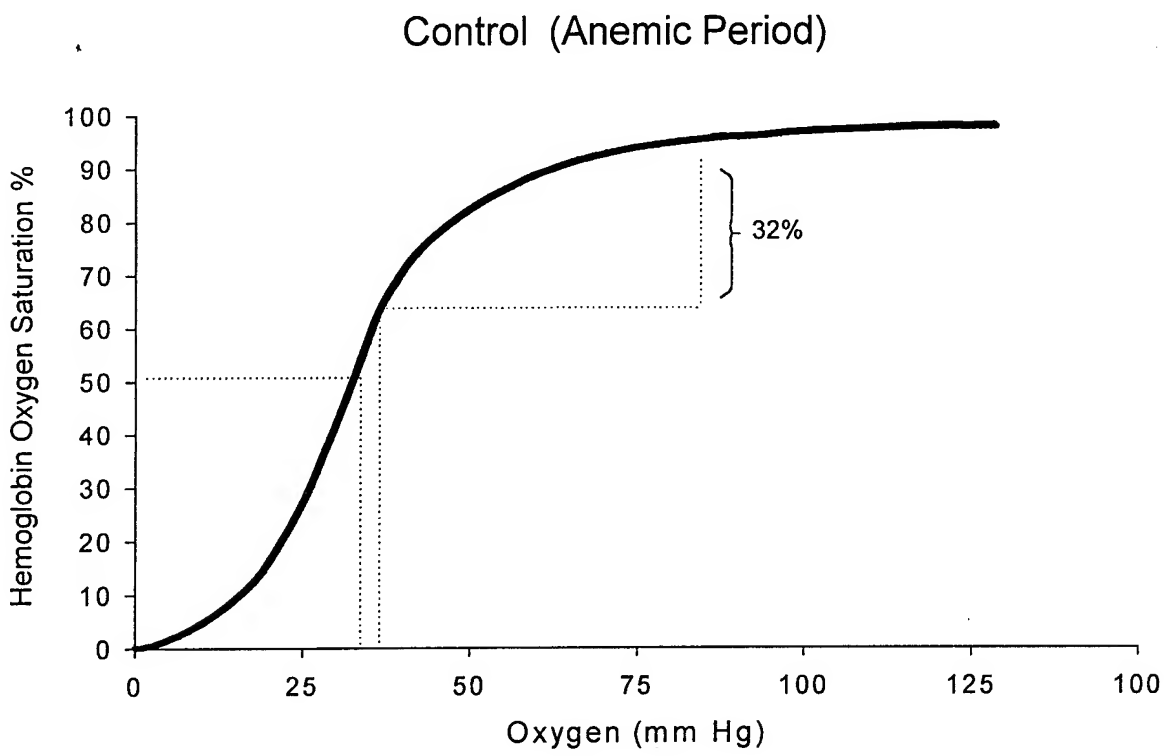
TABLE 4

EFFECT OF PEP BLOOD TRANSFUSION

ON OXYGEN CONSUMPTION

	Pre-Transfusion (cc/kg/min)	Post-Transfusion (cc/kg/min)
Control	4.12 ± .37	3.73 ± .11
PEP	4.88 ± .14	8.31 ± 2.10*

* $P \leq 0.05$, Pre vs Post Transfusion (paired T Test)


Control (Anemic Period)
Hemoglobin Oxygen Saturation %
100
90
80
70
60
50
40
30
20
10
0
32%
0
25
50
75
100
125
100
Oxygen (mm Hg)

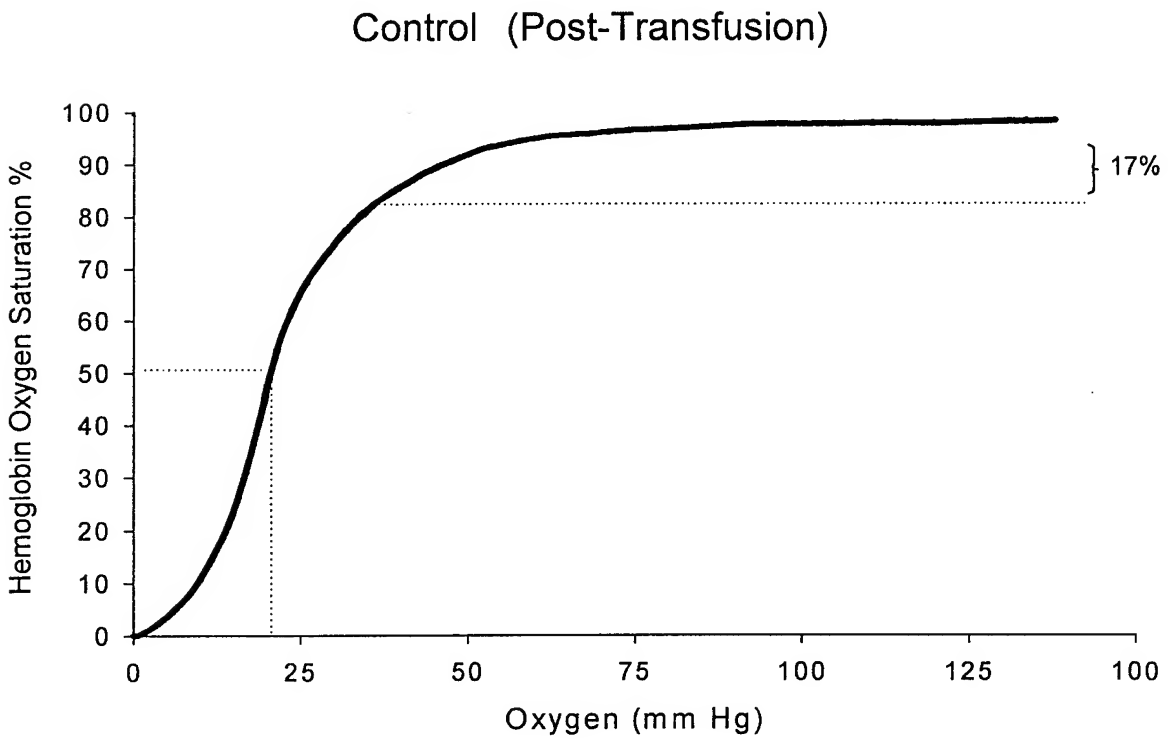
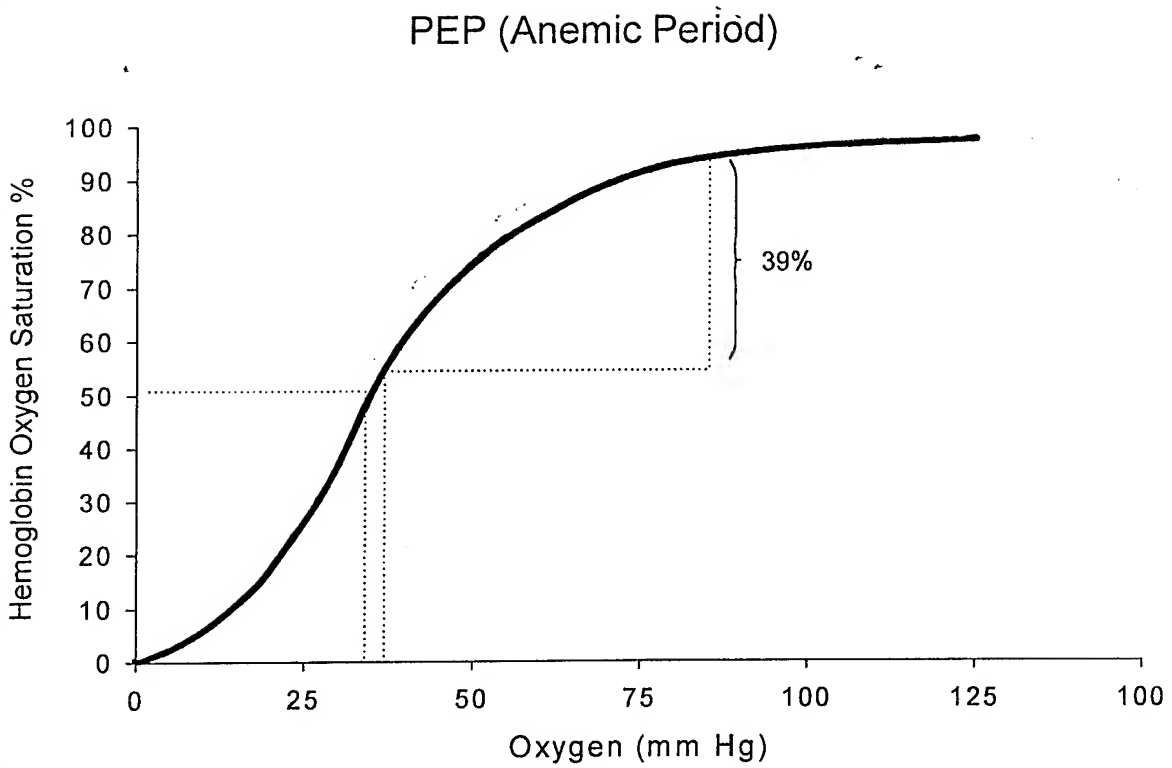

Control (Post-Transfusion)
Hemoglobin Oxygen Saturation %
100
90
80
70
60
50
40
30
20
10
0
17%
0
25
50
75
100
125
100
Oxygen (mm Hg)

Figure 1


PEP (Anemic Period)
Hemoglobin Oxygen Saturation %
100
90
80
70
60
50
40
30
20
10
0
39%
0
25
50
75
100
125
100
Oxygen (mm Hg)

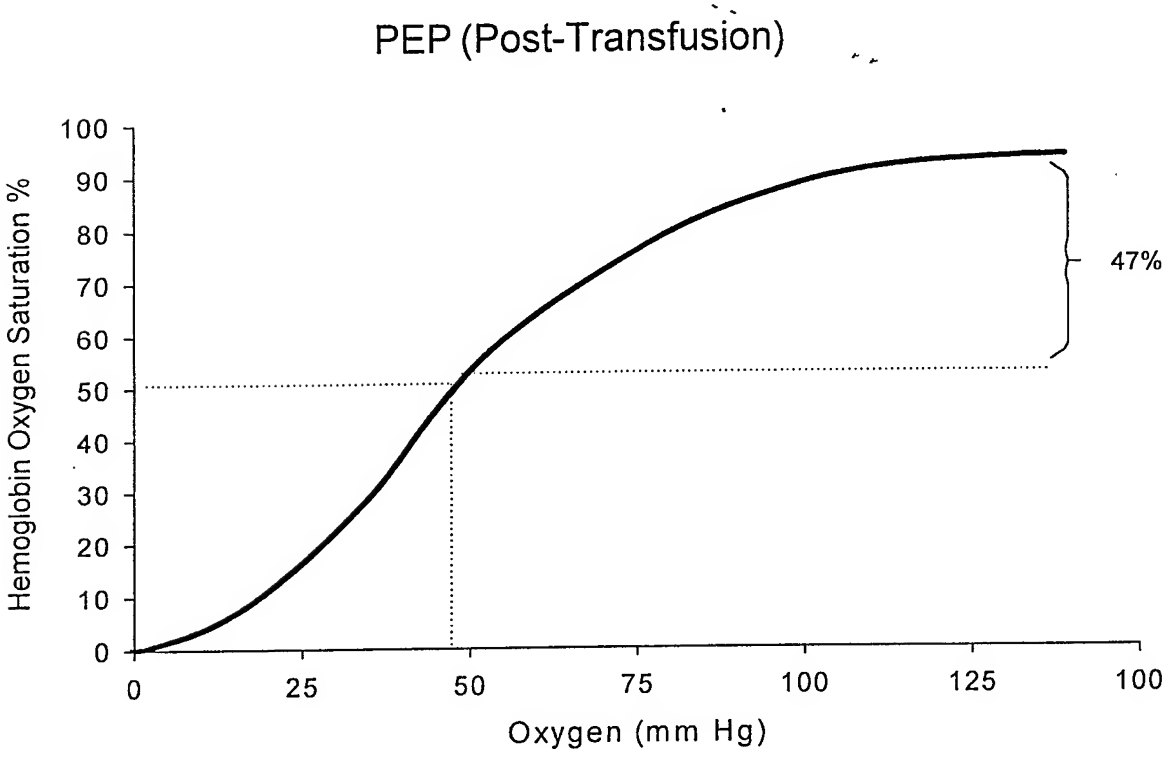

PEP (Post-Transfusion)
Hemoglobin Oxygen Saturation %
100
90
80
70
60
50
40
30
20
10
0
47%
0
25
50
75
100
125
100
Oxygen (mm Hg)

Figure 2